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Measurement and Analysis of Chemically Changed Mineral Fibers after Experiments *in Vitro* and *in Vivo*

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Asbestos, as well as other natural and man-made mineral fibers used for *in vitro* and *in vivo* experiments, must be described and defined physically and chemically as exactly as possible before any application. The interactions of fibers with the physical, chemical (air, water, etc.) and biological (cells, tissues, etc.) environments cause important changes in fiber chemistry and crystalline structure. Also, these should be detected as precisely as possible after each experiment.

Our recent investigations dealt with the development of a complex analytical system for such measurements and with some applications of these analytical procedures for fibrous material sampled in the environment and from biological materials.

Chemical and physical microanalyses of asbestos and glass fibers obtained by environmental sampling (air, water) and from human and animal tissue have shown chemical and crystalline changes in these particles. Scanning electron microscopy, electron microprobe analysis and mass spectrometry analysis were used in these investigations. A partial or total leakage of elements could be observed. The leakage of elements in fibers is of a statistical nature. Some fibers remained chemically unchanged; in some fibers some elements were partially leached; and in some fibers the majority of metallic elements were leached. The potential meaning of this effect is also discussed.

Introduction

It is well known that asbestos fibers (AF) as well as man-made mineral fibers (MMMF) of defined range (concerning fiber lengths L_F as well as fiber diameter D_F—although mechanically stable and insoluble in water—are biologically active in producing lung fibrosis and/or lung cancer and mesothelioma. (*1*). Publications over the last 5 years (*2*) have convincingly demonstrated the primary importance of fiber geometry on the carcinogenic effect. However, this does not exclude chemical properties as well as chemical reactivity of such fibers from being of considerable pathogenic importance (*3-5*).

However, exact (D_F, L_F data and reliable experimental results supporting the limits for fiber geometry have not been obtained, because biological *in vitro* and *in vivo* experiments had not been conducted with fibers of well-defined lengths and diameters. The same is almost true for the chemical composition. The chemistry of fibers (e.g., chemical composition and physicochemical properties of the fiber surface, specific surface, porosity, electric charge, crystal surface structure structure, etc.) has been neglected in the majority of *in vitro* and animal experiments. Often the applied fibrous material is polydispersed and chemically non-homogeneous, or no exact description of fiber geometry and fiber chemical composition is presented. Therefore, any correlation between physical and chemical properties of fibers and the biological effects is very difficult. It must also be considered that interactions with the physical (comminution processes), chemical (air, rain, water) and biological (cells, tissues) environments cause important changes in fiber size, chemistry and crystalline structure.

Any biological experimental investigations on fiber activities should be done with fibers which have been physically and chemically defined. Our investigations of the last few years (*6-10*) are dealing with the development of physical and chemical methods for the preparation of size-selected fiber fractions as well as with the development of a complex analytical system for exact description of fiber composition and of chemical changes in fibers after biological ap-

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plications and in tissues. The main results obtained to date are described and discussed in this short review.

Materials and Methods

UICC Standard Reference Asbestos (11) and Johns Manville glass fibers (MN 100 to 106) were used in these studies.

Fiber Sizing

When inhalation experiments or instillation applications are to be done on animals, the first requirement is to have an inhalable fiber fraction for disposition. In such cases an application of the previously described vibrating bed aerosol generator (8) was successful. The generator (Fig. 1) provides relatively good constancy of the fiber concentrations (range $\leq$ 20 mg/m^3) and fiber size distribution. The principle is based on a vibrating fluidized bed. The powder (consisting of fibers or of isometric particles) to be used for generating the dust cloud is placed in a vibrating cylinder, and clean, dried gas is passed vertically through the powder layer to form a fluidized bed. The vibrations destroy the cohesion forces be-

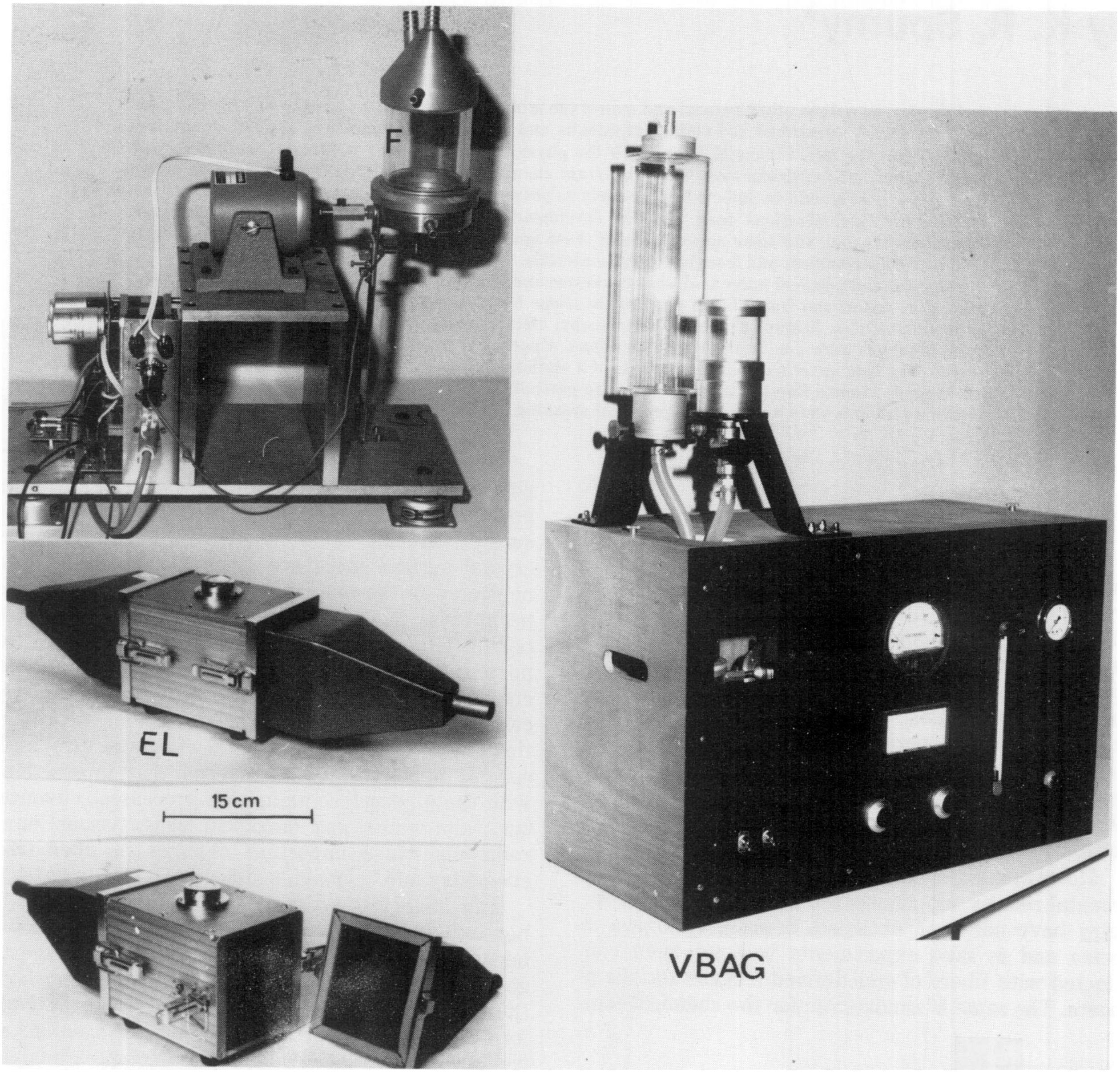


FIGURE 1. Portable vibrating bed aerosol generator (VBAG) and its accessories. The powered material is fluidized in the cylinder F, which is coupled to a vibrator. A sedimentation elutriator EL is used for selecting respirable fractions.

tween particles or fibers, which become free and can be carried away by the gas stream.

It is possible in this generator to control the aerosol concentration and particle size distribution quite closely by adjusting the vibration frequency and amplitude. The optimum frequency range for the release of fibrous particles has been found to be about 15 to 50 Hz in the amplitude range 100 to 1000 μm. For each powdered material the dependency between particle size distribution (concentration), vibration frequency, and amplitude must be determined and calibration curves drawn.

This equipment is very suitable for the preparation of inhalable or respirable particle or fiber fractions as well as aerosol (*6*). This generator can be used to pulverize coarse dispersed powders (quartz, coal dust, cement, asbestos-cement dust, fibrous powders, etc.) and to separate the nonrespirable fraction by means of an elutriator (Fig. 1) combined in series with the aerosol output. The respirable powder or fiber fractions can then be used as an inhalation aerosol for animals, or this fraction can be sampled on a membrane filter and then used for instillation or *in vitro* experiments. As much as 50 to 200 mg of these fine fractions can be produced by this method in 8 hr.

A small portable vibrating bed aerosol generator of this type is now commercially available (Firma G. WAZAU, Kepler Str. 12, D-1000 Berlin 10).

The preparation of size-selected inorganic fibers for intratracheal and intraperitoneal applications is a more difficult problem. We have described some attempts for such fractionations and we have also successfully produced a few grams of size-selected asbestos and glass fibers that were used in *in vitro* investigations (*12*). Nevertheless, more investigations are needed in this area to perfect all steps in such a procedure.

The fiber size reduction was done by a chopping procedure. In the chopping apparatus, dry fibers were cut by a rapidly rotating set of knives.

For the separation of fibers by diameter, a repetitively applied sedimentation or centrifugation of liquid suspensions was the most effective method. The behavior of asbestos fibers in a liquid and some of their colloid-chemical, as well as surface, properties have already been described and could be exploited. Sieving was then used for the selection of fibers by length. Fibrous aerosols prepared by means of the described generator or fiber suspension in very pure distilled water were fractionated by repeated sieving. Vibration of polymer microsieves (mesh diameters 5 to 50 μm) at 100 Hz could be used successfully. By repeated screening, the fibrous material was fractionated by length and then, by repetitive settling in liquids (constant temperature), a separation by fiber diameter was achieved. Examples of some results obtained by means of this procedure are demonstrated in Figure 2.

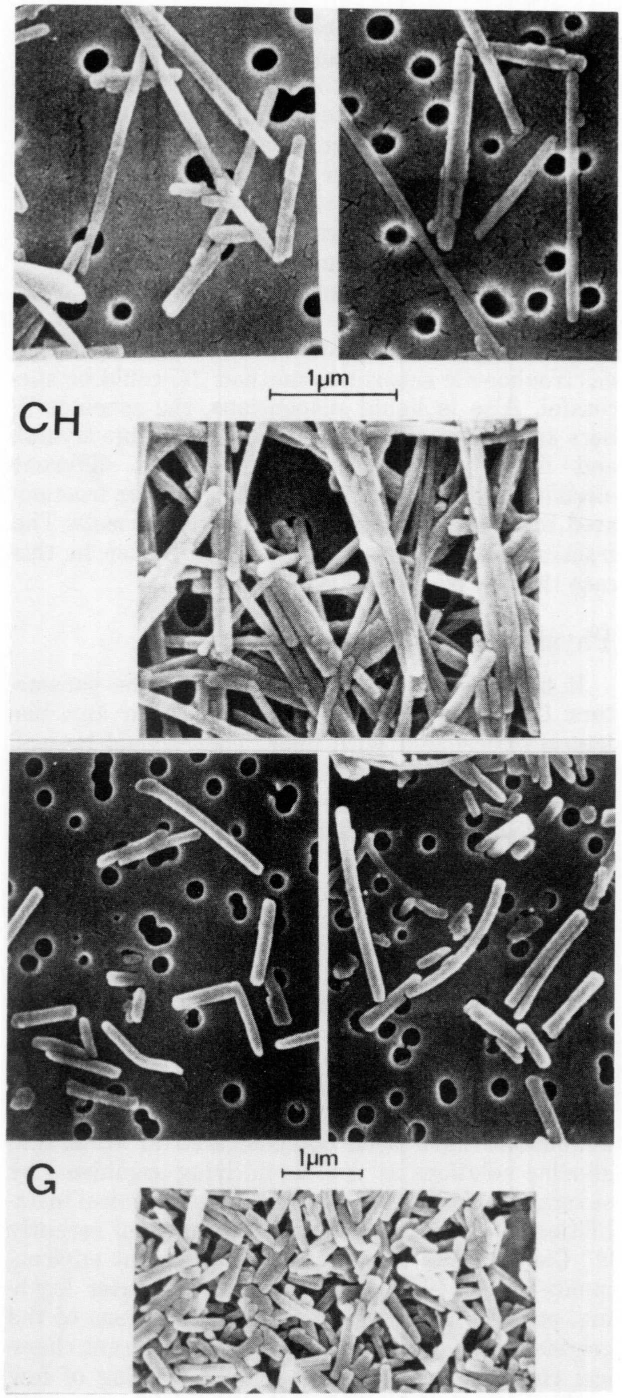


FIGURE 2. Size-selected fiber fractions. Scanning electron micrographs of chrysotile fibers (CH) and glass fibers (G) with $L_F \lesssim 3$ μm and with a very narrow D_F distribution.

More successful and more precise fractionation by fiber length was observed by applications of electrostatic fields (*13*). An electrically charged fibrous aerosol prepared by means of the described generator was fractionated in an asymmetric cylindrical electrostatic field. The aerosol flowed through an electrical aerosol charger. Charged fibers (the electric charge of each fiber is approximately proportional to the L_F were then fractionated by their mobilities in the electric field (mobility spectrometer) and separated on a Millipore or Nuclepore filter. The production output is small—1 mg in 24 hr.

Preliminary experiments have also shown that an electrophoretic separation method (*14*) could be successful. Also in liquid suspensions, the asbestos fibers are electrically charged (electric double layers), and fibers of different sizes possess different electrical mobilities. They can therefore be fractionated in a similar way as the fibrous aerosols. The fraction outputs probably could be better in this case than by fractionation of aerosols.

Physical Description

It has already been mentioned that the parameters L_F D_F have a basic importance to any biological experiment with fiber. They should be well defined before a model fiber type is used. The best description is a size distribution of both parameters or a two-dimensional distribution: relative fractions as a function of D_F and L_F. Also of importance is the crystalline structure of the fibrous material, which can be well evaluated by means of X-ray diffraction. Examples of such a physical description of fibers for applications in biological experiments are demonstrated in Figure 3 and Table 1.

Chemical Description

Chemical instabilities of chrysotile and of some amphiboles have been demonstrated in acidic and alkaline solutions as well as in living organisms by several researchers (*2, 3*). Also some chemical instabilities of glass fibers have been reported recently (*9*). Chrysotile is also unstable in different environments (air corrosion and weathering, water leaching, etc.). To describe chemical compositions of the original model fibers as well as their different chemical changes, a complex method consisting of few modern physical analytical methods (*15*) was used. Qualitative changes in the surface structure of different fiber types can be observed from scanning electron microscopy. (SEM) investigations (Fig. 4), and the specific surface area can be well measured by the BET method. By means of multi-element X-ray fluorescence-spectroscopy, elemental analysis of bulk fiber (original material as well as fibers after exposure in tissues or liquids) can be done (Fig. 5). Transmission electron microscopy (TEM) with three possible identification methods—EDXA, SAED and morphological observations—is a very useful tool for observation and analysis of single fibers (Fig. 6). The combination of SEM + EDXA offers a lower analytical sensitivity than TEM + EDXA, but it makes possible to analyze fibers directly in tissue slices (Fig. 7).

By means of a new and very sensitive method—mass spectroscopic analysis of single fibers (the LAMMA procedure)—a microchemical analysis of original as well as of chemically changed single fibers can be done. The instrument used is a laser microprobe mass analyzer, a combination of a laser beam of high energy with a special mass spectroscope. The principle on which LAMMA is based is the excitation of a microvolume of the sample to an ionized state by a focused laser beam. The analytical information is derived from mass spectroscopy of these ions. It is obvious that all elements of the periodic table and their isotopes can be detected. The LAMMA spectra of positive and negative ions contain much information about atomic and molecular constituents of single particles or single fibers. In addition the sensitivity is high enough to permit detection of trace contaminants in the ppm or ppb range, and the measurement and data processing are fast enough to allow for the collection of statistically significant information of the particle or fiber population as a whole (*16*). Mass spectra of asbestos (Fig. 8), man-made mineral fibers (Fig. 9), as well as of other mineral fibers (Fig. 10) obtained by LAMMA, are very reproducible and characteristic of each fiber type. A very good differentiation could be found between serpentines and amphiboles. Also, a differentiation between amosite and crocidolite seems to be possible. LAMMA spectra can be considered as a good fingerprinting for different asbestos minerals, glass fiber and other natural and man-made mineral fibers. The first applications of the LAMMA instrument to fiber research showed that this new microprobe can give very useful information concerning the chemical nature as well as the chemical changes of the single fiber material (9).

One other very important method for analysis of chemical composition of mineral fibers is Auger spectroscopy (X-ray photoelectron spectroscopy) ESCA, or scanning auger microscopy (SAM). This method is used to measure the kinetic energy of electrons photo-ejected from a surface using soft X-rays or electron beams. The approximate escape depth from silicates is about 7.5 nm. This method is sensitive to all elements with an atomic number higher than 2. The method can be used very successfully for analysis of chemical changes of fiber surfaces (*2*).

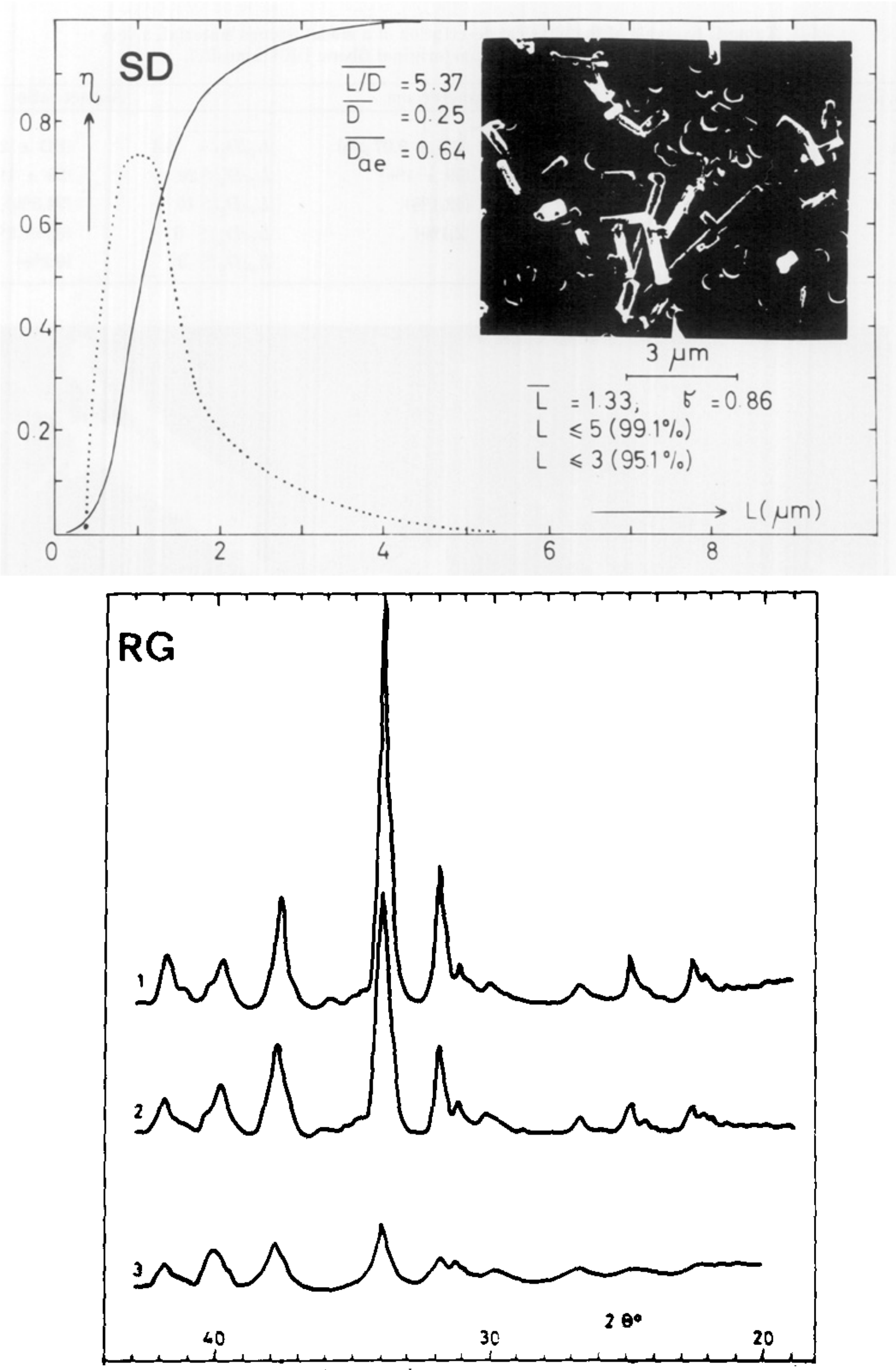


FIGURE 3. Example of the physical description of model fibers: (*SD*) Scanning electron micrograph and the fiber length distribution (*L*) of a "short" amosite asbestos sample; (RG) X-ray diffraction spectra of amosite fibers with different fiber sizes as indicators of degradation of the crystalline structure (1) original UICC amosite sample, (2) amosite with $L_F \leq 3$ μm and (3) amosite ground in a ball mill for 48 hr.

Table 1. Example of the physical description of a model fibrous material, a very fine amosite with $L_F \leq 3$ μm (original fibers: UICC amosite).

Fiber Length, μm		Fiber diameter, μm		Aspect ratio	
$\overline{L_F}$ = 1.17 μm	(SD ± 0.47)	$\overline{D_F}$ = 0.23	(SD ± 0.07 μm)	$\overline{L_F/D_F}$ = 5.4	(SD ± 2.41)
$L_F \leq 3.00$ μm	(99 ± 1%)	$D_F \leq 0.5$	(99 ± 1%)	$L_F/D_F \leq 20$	(99 ± 1%)
$L_F \leq 1.00$ μm	(48.3%)	$D_F \leq 0.2$	(42.1%)	$L_F/D_F \leq 10$	(94.3%)
		$D_F \leq 0.1$	(2.1%)	$L_F/D_F \leq 5$	(62 61.2%)
				$L_F/D_F \leq 3$	(6.2%)

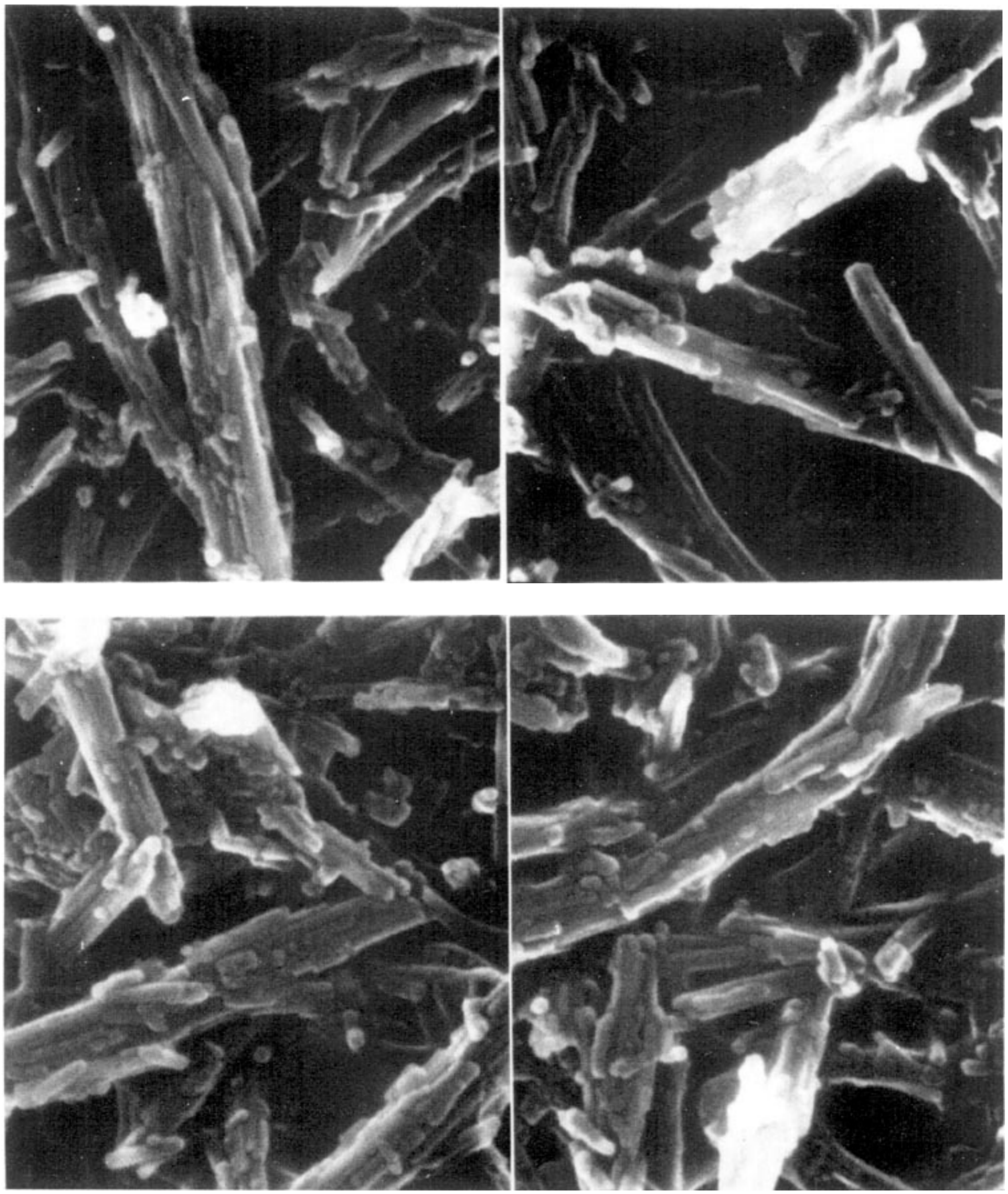


FIGURE 4. Scanning electron micrographs of chrysotile fibers after 1 month exposure to 2 N HCl. Morphological degradation is evident.

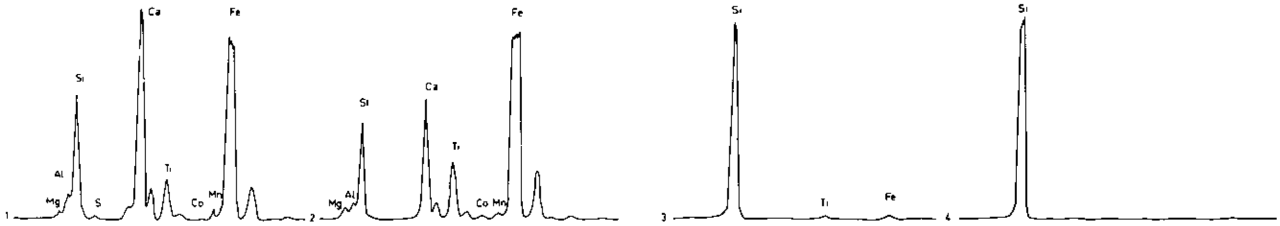


FIGURE 5. X-ray fluorescence-spectra of small samples of rock wool fibers after long-term exposure in (1) H_2O, (2) 2 N NaOH, (3) 2 N HCl and (4) 2 N H_2SO_4. Leaching of elements and chemical degradation are well demonstrated.

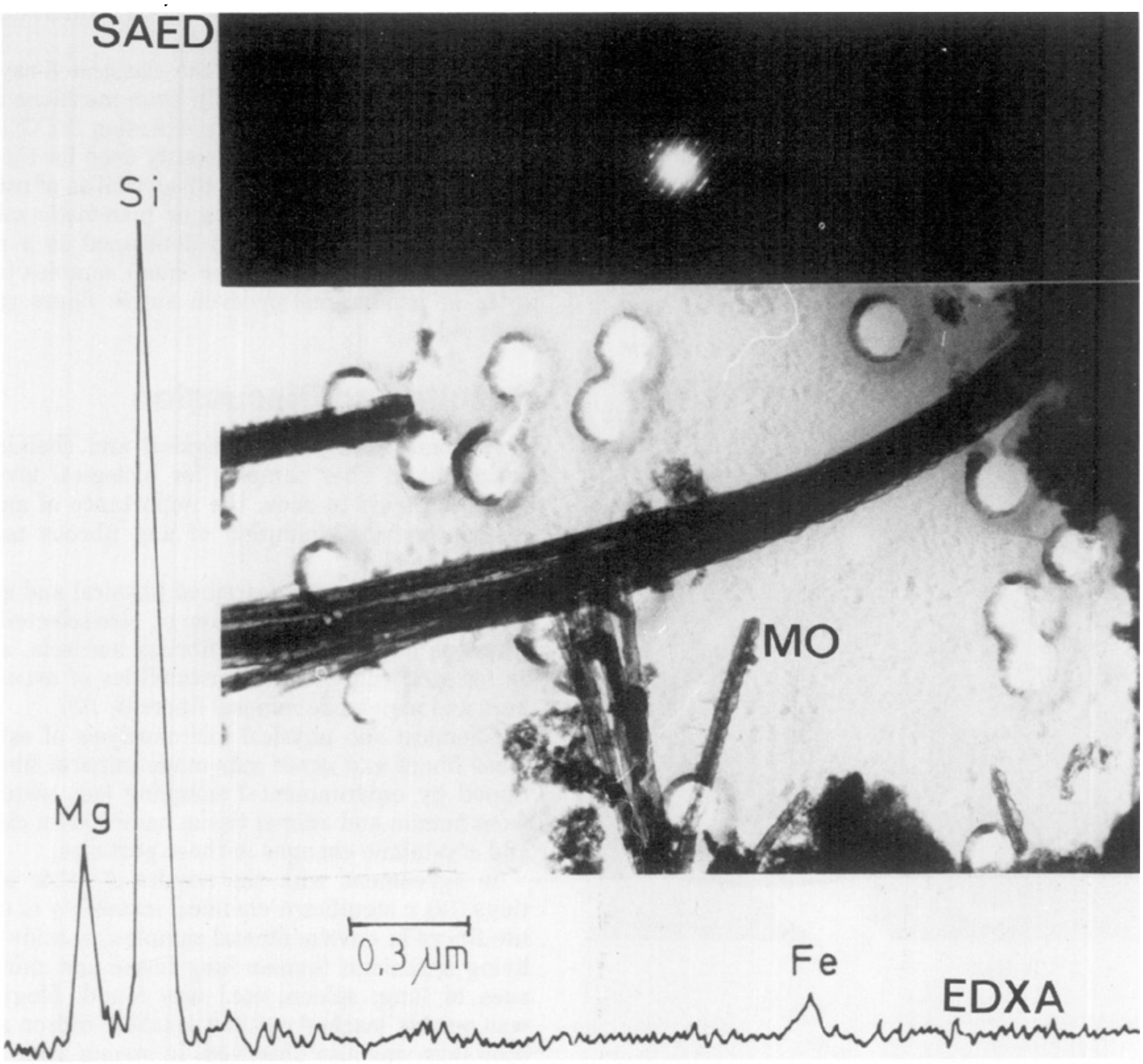


FIGURE 6. Chemical, morphological and crystallographic analysis of chrysotile fibrils showing EDXA (energy-dispersive X-ray fluorescence spectroscopy), SAED (selected-area electron diffraction) and MO (morphological observations).

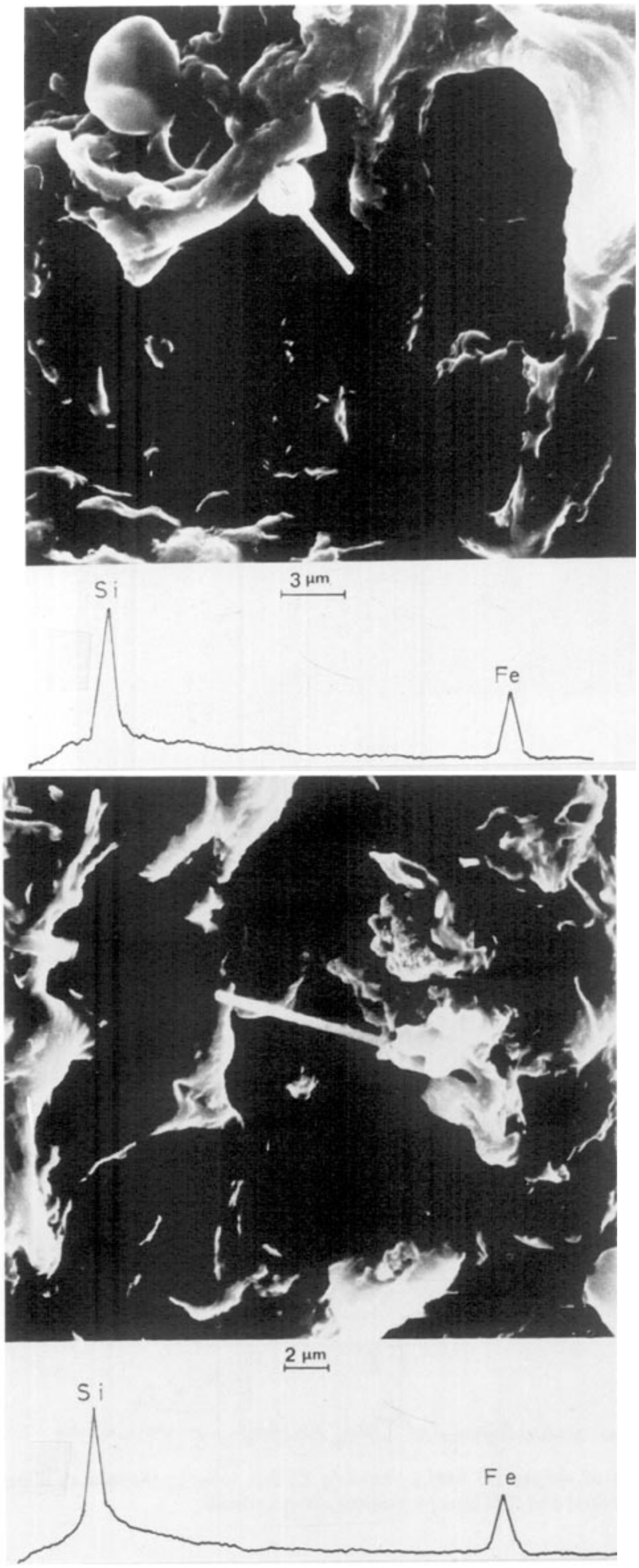


FIGURE 7. Electron micrographs (SEM) and EDXA analysis of typical single asbestos fibers in human lung tissue.

An example of such an analysis is shown in Figure 11. The method can be used for quantitative analysis as well.

Classical Raman spectroscopy has been also developed for analysis of single particles (Raman microprobe) (*17*). In this case the sample is illuminated by a laser beam, causing characteristic Raman lines of the different components to be emitted. With these Raman lines, the components can then be detected, identified and located. One main advantage of this technique is the ability to study samples in air rather than under vacuum. Normal Raman spectra can be obtained from particles or fibers of about 1 µm size and larger. Examples shown in Figure 12 demonstrate a good differentiation between chrysotile and amosite.

A suitable application of the classical X-ray fluorescence analysis has already been mentioned (Fig. 5). The proton-induced X-ray exitation (PIXE) techniques can also be advantageously used for the evaluation of dust samples (Fig. 13) as well as of samples of different fibrous minerals or man-made mineral fibers. PIXE had been also developed as a microprobe analysis and therefore small samples (of the order of nanograms) or even single fibers can be analyzed (*18*).

Results and Discussion

In some examples of physical and chemical descriptions of fiber samples for biological investigations, we tried to show the importance of an exact description and definition of any fibrous material that is used.

We have used the described physical and analytical methods for preparation of size-selected fiber fractions and well-defined fibrous aerosols, as well as for studying chemical instabilities of asbestos fibers and man-made mineral fibers (*9*, *10*).

Chemical and physical microanalysis of asbestos, glass fibers and other man-made mineral fibers obtained by environmental sampling (air, water) and from human and animal tissue have shown chemical and crystalline changes in these particles.

In agreement with the results of other publications (*19*), a significant chemical instability of chrysotile fibers in environmental samples, in acids and in living organisms (human lung tissue and animal tissues of lung, spleen, etc.) was found. Magnesium was quickly leached out, but leaching of iron and silocon ions was also observed. In strong acids, entire magnesium hydroxide sheets of chrysotile were stripped away, leaving a silica skeleton behind. Results of analysis of about 300 chrysotile fibers (human lung tissue and UICC chrysotile after extraction in acids) examined by LAMMA indicated

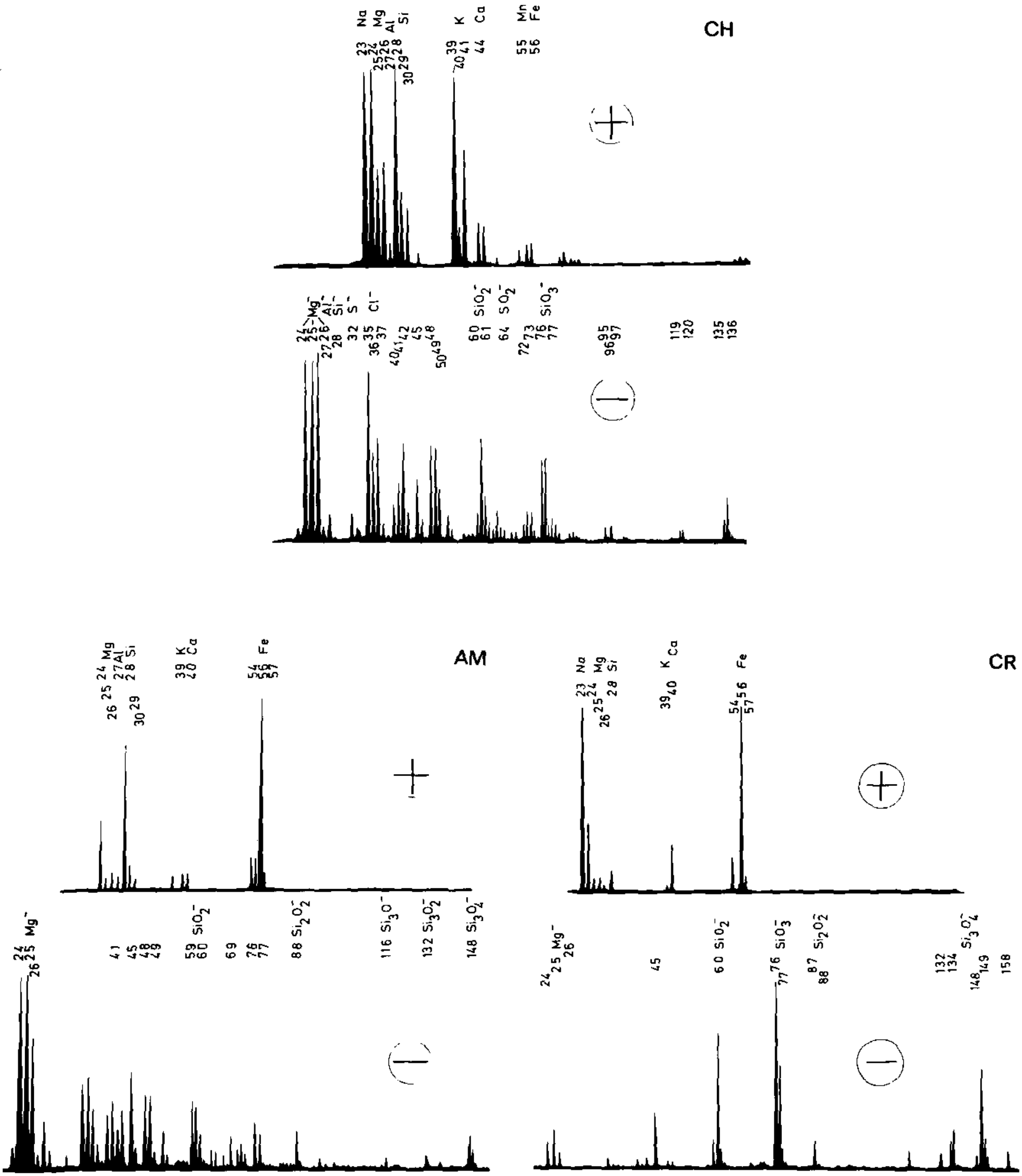


FIGURE 8. *LAMMA spectra for positive (+) and negative (−) ions obtained from UICC asbestos fibers:* (CH) chrysotile, (AM) amosite and (CR) crocidolite.

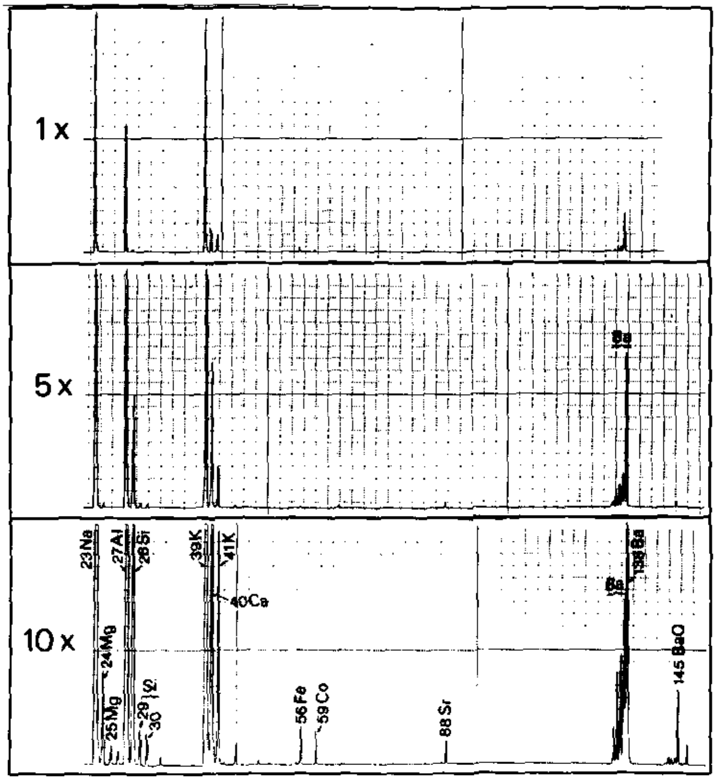


FIGURE 9. LAMMA spectra of positive ions obtained from JM 106 glass fibers at different sensitivities (1 to 10).

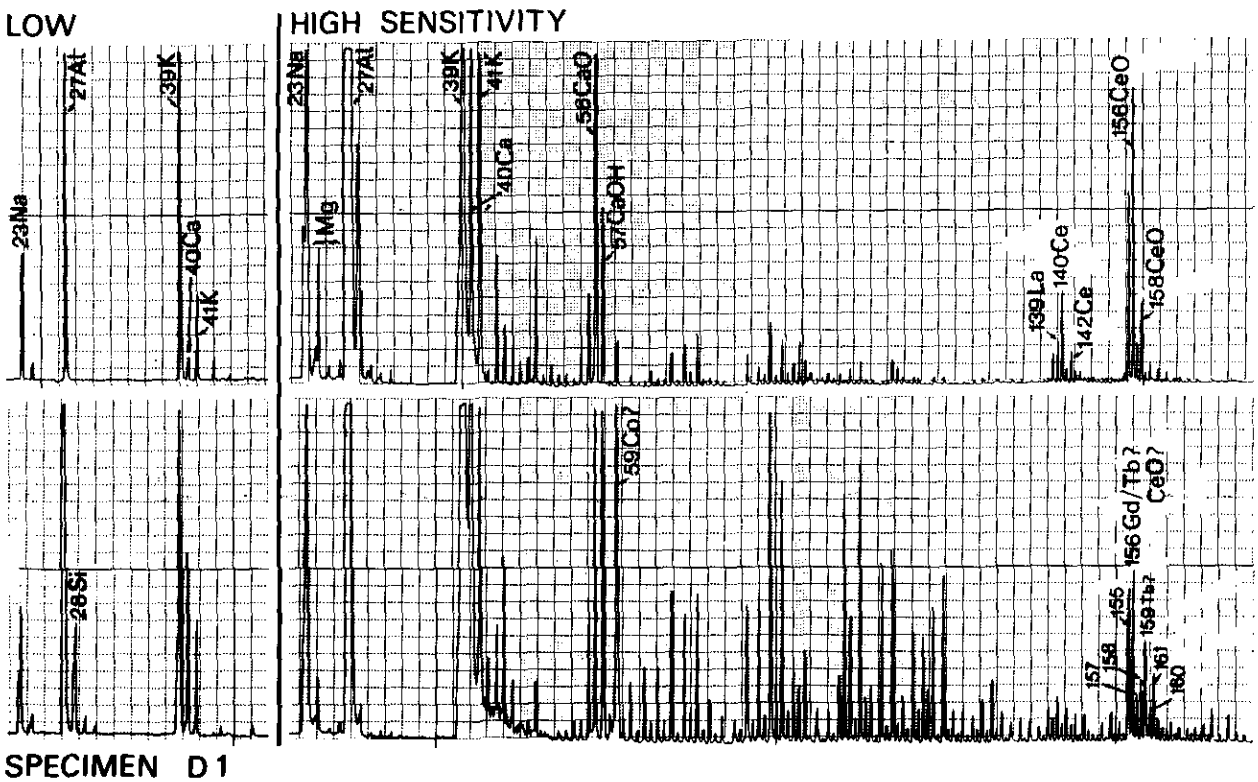


FIGURE 10. LAMMA spectra for positive ions obtained from a fibrous erionite (zeolite) having fiber diameters of 1-2 μm and fiber lengths of 10-15 μm.

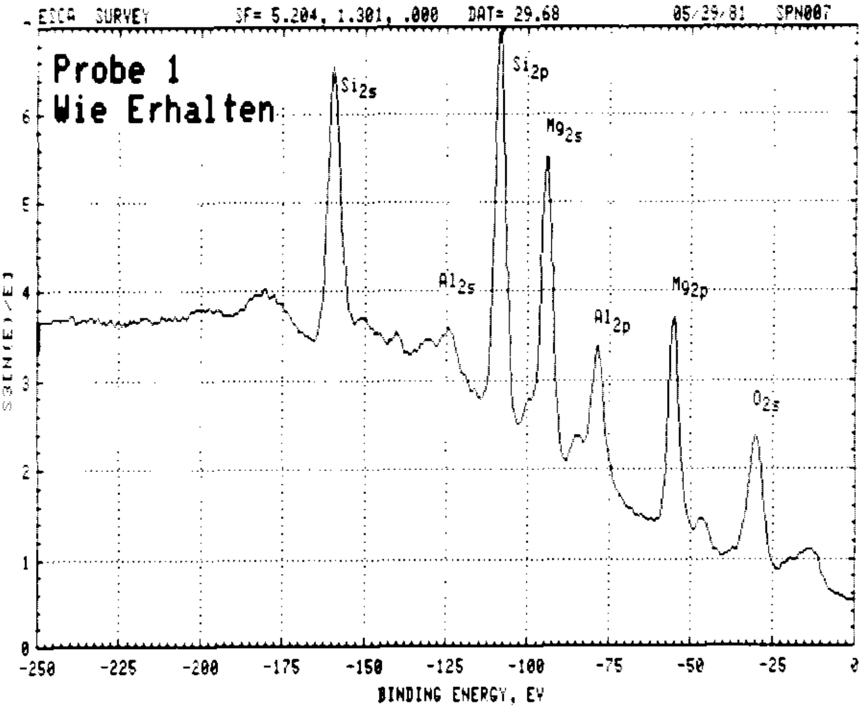
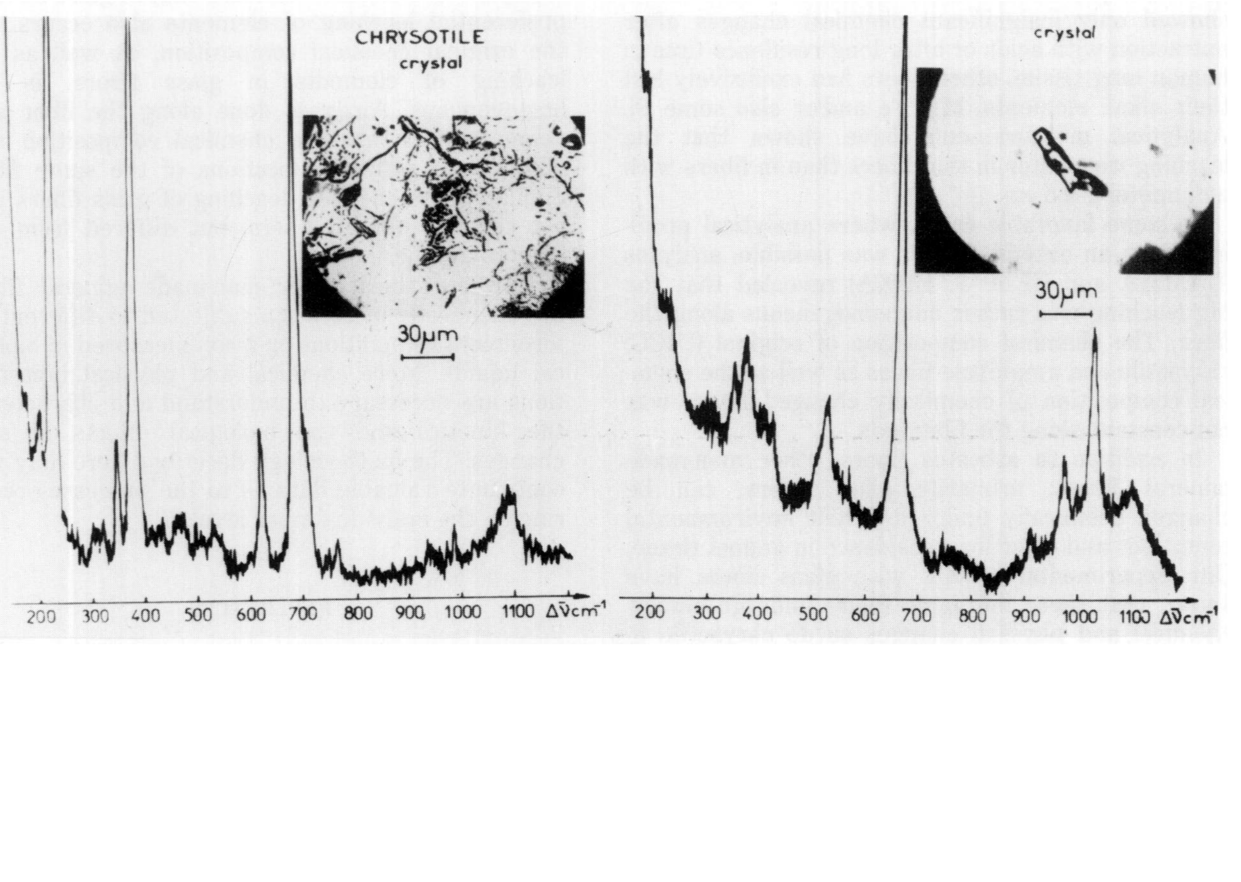


FIGURE 11. Auger element spectrum obtained from UICC chrysotile fibers.



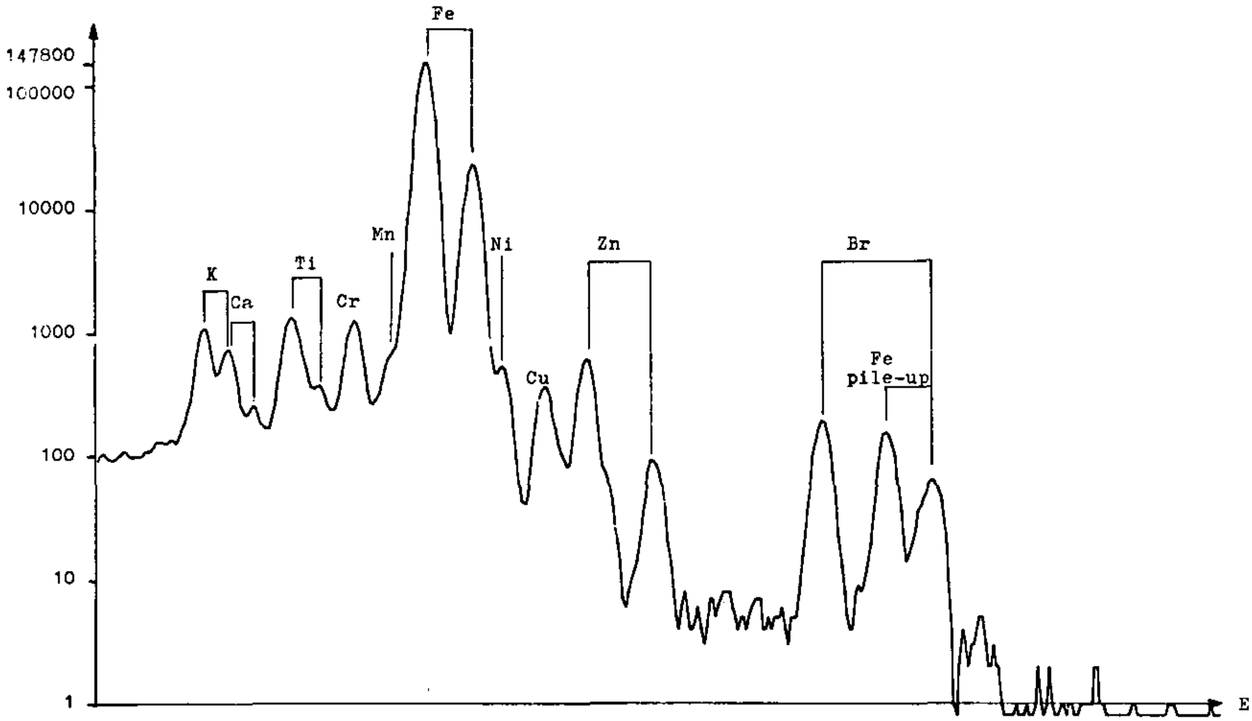


FIGURE 13. PIXE X-ray spectrum from lung dust (asbestosis). (Energy $E_p = 2$ MeV, charge 1.1 μC).

that the degree of corrosion (leaching of Mg, Fe and Si) in single fibers is statistically distributed in a larger fiber population. While some chrysotile fibers showed only insignificant chemical changes after extraction with acids or after long residence time in human lung tissue, other fibers had exclusively lost their alkali elements, Mg, Fe and/or also some Si. Analytical measurements have shown that the leaching was faster in thin fibers than in fibers with a diameter ≥ 1.5 μm.

In some favorable cases, where analytical profiling along an extended fiber was possible, analyses (LAMMA and TEM + EDXA) revealed that the Mg leaching was rather nonhomogeneous along the fiber. The chemical composition of original (UICC) chrysotile and amphibole fibers as well as the chemical composition of chemically changed fibers was not constant along the fiber axis.

In addition to asbestos fibers, other man-made mineral fibers, primarily glass fibers, can be changed chemically under different environmental conditions and after long residence in animal tissue. Our experimental results with glass fibers have shown that these inorganic fibers undergo similar chemical and physical changes as do chrysotile fibers. They are not resistant to basic solutions. They lose some elements (e.g., Na, K, Ca, Al, Zn) upon exposure to acids, and their surface becomes corroded. Changes of chemical composition have been found in glass fibers after 5 years deposition in animal tissue (instillation experiments done on rabbits). Analysis by LAMMA revealed that in glass fibers a preferential leaching of elements also occurs, but the original chemical composition, as well as the leaching of elements in glass fibers, is not homogeneous. Analyses done along the fiber axis demonstrated that the chemical composition was different at different locations of the same fiber. Similarly, the chemical leaching of glass fibers was not constant for all fibers, but differed from one fiber to another.

Neither asbestos nor man-made mineral fibers are chemically inert when subjected to different environmental conditions or when measured in biological liquids. More chemical and physical investigations are necessary to understand and characterize the kinetics and the biological effects of such changes. The methodology described here may well contribute valuable data as to the processes occurring on the individual fiber level.

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